ChemComm
www.rsc.org/chemcomm

Amino acid functionalisation of water soluble carbon nanotubes

Vasilios Georgakilas,[a] Nikos Tagmatarchis,[a] Davide Pantarotto,[a,b] Alberto Bianco,*[b] Jean-Paul Briand[b] and Maurizio Prato*[a]

[a] *Dipartimento di Scienze Farmaceutiche, Università di Trieste, 34127 Trieste, Italy. E-mail: prato@units.it*

[b] *Institut de Biologie Moléculaire et Cellulaire, UPR9021 CNRS, 67084 Strasbourg, France. E-mail: A.Bianco@ibmc.u-strasbg.fr*

Received (in Cambridge, UK) 8th October 2002, Accepted 29th October 2002
First published as an Advance Article on the web 14th November 2002

High solubility of SWNTs and MWNTs in water is obtained by organic functionalisation; derivatisation with N-protected glycine is also easily achieved.

Due to their exceptional combination of mechanical, thermal, chemical, and electronic properties,[1] single-wall (SWNT) and multi-wall carbon nanotubes (MWNT) are considered as unique materials, with very promising future applications, especially in the field of nanotechnology,[2] nanoelectronics,[3] composite materials[4] and medicinal chemistry.[5]

So far, potential biological applications of carbon nanotubes (NT) have been very little explored.[5] Biosensors based on SWNTs and MWNTs have been developed by incubating proteins with the tubes either *via* non-covalent or covalent binding.[5a–d] Antibodies generated against fullerenes are able to specifically recognize SWNTs thus opening the way to the use of these systems as probes of cell or membrane function.[5e] However, a limitation to the biological applications of NT is certainly due to the difficulty in solubilizing such materials in aqueous solutions as a part of their biocompatibility.

Solubilisation of NTs in organic solvents has been described in the literature,[6] mainly based on the attachment of building blocks to the carboxylic functions that are formed by oxidation of NTs using strong acids.[6,7] This method has the disadvantage of cutting the NTs in short pieces of about 100–300 nm, making them not useful for applications that are based on their length. Soluble NTs can also be obtained by polymer wrapping.[8]

Soluble full-length NTs have been recently achieved by side-wall organic functionalisation.[9,10] This type of solubilisation makes their manipulation and incorporation in different materials easier.

To extend the applications of NTs in medicinal chemistry, water soluble samples are in demand. Very recently, it has been shown that NTs can be solubilised in aqueous solution by a wrapping approach using starch[11] and poly(vinylpyrrolidone)[12] or attaching monoamine terminated poly(ethylene oxide),[13] glucosamine[14] or crown ethers[15] to the carboxylic groups of the oxidized SWNTs.

In this paper, we describe the solubilisation in aqueous media of side-wall chemically modified, full length SWNTs and MWNTs and their derivatisation with N-protected amino acids. The method of functionalisation is based on the 1,3-dipolar cycloaddition reaction to the external surface of the nanotube.[10] The SWNTs and MWNTs (diameter: 20–30 nm) used in this work were obtained from Carbon Nanotechnologies, Inc., USA (www.cnanotech.com), and Nanostructured & Amorphous Materials, Inc., USA (www.nanoamor.com), respectively.

Amino acid **1**[16] and paraformaldehyde were added to a suspension of NTs in dimethylformamide (DMF) and the mixture was heated at 130 °C for 96 h (Scheme 1). After separation of the unreacted material by filtration, followed by evaporation of the solvent, the resulting residue was diluted with chloroform and washed with water. The combined organic phases were dried and the solvent was evaporated. Functionalised NTs **2** were isolated by precipitation with diethyl ether and the solid was subsequently washed several times with diethyl ether. The yield, based on the amount of starting SWNTs was about 10%. The final material was found to be soluble in most common organic solvents such as acetone, chloroform, dichloromethane and toluene.

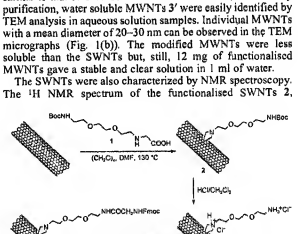
To a solution of functionalised NTs **2** in dichloromethane (DCM), gaseous HCl was bubbled to remove the N-*tert*-butoxycarbonyl protecting group (Boc) at the chain-end. The corresponding NT ammonium chloride salt **3** precipitated during the acid treatment. After removal of the solvent, the brown solid was dissolved in methanol and precipitated with diethyl ether.

SWNTs **3** possess a remarkably high solubility in water. In fact, 20 mg of **3** gave a stable solution in 1 ml of water for more than a month.

Analysis of **3** by transmission electron microscopy (TEM) showed the presence of SWNTs in the water solution (Fig. 1(a)). The SWNTs appear in bundles with diameters in the range 10–50 nm. Based on the TEM images, a significant purification of the material can also be observed, since HiPCO tubes contain a remarkable amount of metal nanoparticles.

The same 1,3 dipolar cycloaddition reaction, using the same reagents as shown in Scheme 1, was also performed with MWNTs. Functionalised MWNTs **2′** showed a ^{1}H NMR very similar to that of SWNTs **2**. After acid treatment, followed by purification, water soluble MWNTs **3′** were easily identified by TEM analysis in aqueous solution samples. Individual MWNTs with a mean diameter of 20–30 nm can be observed in the TEM micrographs (Fig. 1(b)). The modified MWNTs were less soluble than the SWNTs but, still, 12 mg of functionalised MWNTs gave a stable and clear solution in 1 ml of water.

The SWNTs were also characterized by NMR spectroscopy. The ^{1}H NMR spectrum of the functionalised SWNTs **2**,



Scheme 1

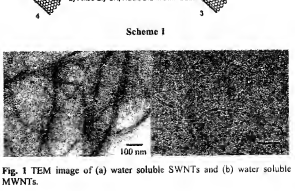


Fig. 1 TEM image of (a) water soluble SWNTs and (b) water soluble MWNTs.

DOI: 10.1039/b209843a

confirmed the presence of the functionalised oligoethylene glycol chain as a broad peak at 3.6 ppm and the methyl protons of Boc group at 1.2 ppm. The latter signal disappeared after treatment with HCl.

Following the cleavage of Boc group, the amount of amine functions was determined by a quantitative Kaiser test.[17] The loading of functionalised carbon nanotubes was calculated in the range between 0.25–0.50 mmol per g of material.

The free amino groups can be easily derivatised with N-terminal protected amino acids. Indeed, N^{15}-labelled Fmoc-Gly-OH was activated with *N*-hydroxybenzotriazole (HOBt) and diisopropylcarbodiimide (DIC) in DMF–DCM for 15 min and added to a suspension of **3** in DCM, previously neutralised with diisopropylethylamine (DIEA). After stirring at room temperature for 2 h, the coupling reaction was terminated (negative Kaiser test)[18] and the solvent was completely evaporated. The raw material was dissolved in DCM and the derivatised NTs were reprecipitated several times by addition of diethyl ether. Nanotubes **4** were structurally characterized by TEM microscopy and NMR spectroscopy.

TEM micrographs were very similar to those reported in Fig. 1, thus confirming the nanotube structure of **4**. The ^{1}H NMR spectrum of **4** in acetonitrile shows the presence of the oligoethylene glycol chain at about 3.5 ppm as a broad peak. The aromatic protons of Fmoc group are located at 7.3–7.8 ppm, while the methylene and methine signals of Fmoc are present around 4.2 ppm (Fig. 2). The α-CH_2 protons of Gly are partially overlapped with the $-OCH_2-$ signal at about 3.8 ppm. It is difficult to assign the resonances of pyrrolidine protons due to the different local magnetic environments on the NT surface. The broad signals at 2.9 and 8.0 ppm are attributed to the methyl and amide protons of DMF, that remains trapped within the nanotubes to a certain extent even after extensive washings.

The presence of the N^{15}-labelled nitrogen on the Gly residue allowed us to perform a DEPT45 experiment[19] (Fig. 2, inset). The single peak measured at −315.57 ppm in CD_3CN (referenced to the external standard nitromethane) is indicative of a homogeneous distribution of the N-protected amino acid around the nanotube side-wall.

In conclusion, we have successfully generated amine functionalised and water soluble SWNTs and MWNTs that can be easily derivatised with N-protected amino acids. This is the first step towards the synthesis of peptide-based carbon nanotubes. We are currently exploring the possibility of preparing SWNTs decorated with covalently attached peptides for both conformational and biological studies.

This work was carried out with partial support from the European Union, Human Potential Network FUNCARS (HPRN-CT-1999-00011), the University of Trieste (Fondi ex 60%) and MIUR (cofin prot. MM03198264). The authors thank Mr Claudio Gamboz and Prof. Maria Rosa Soranzo (CSPA, University of Trieste), for kind help with TEM analysis, Dr Ken Smith at Carbon Nanotechnologies, Inc., for a generous gift of HiPCO nanotubes, Dr Fabio Hollan (CSPA, University of Trieste) for mass spectra and Dr Roland Graff of the NMR facilities at the University Louis Pasteur of Strasbourg for recording the NMR data.

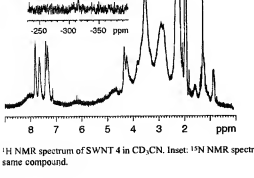


Fig. 2 ^{1}H NMR spectrum of SWNT **4** in CD_3CN. Inset: ^{15}N NMR spectrum of the same compound.

Notes and references

1 (*a*) C. N. R. Rao, B. C. Satishkumar, A. Govindaraj and M. Nath, *ChemPhysChem.*, 2001, **2**, 78; (*b*) E. W. Wong, P. E. Sheehan and C. M. Lieber, *Science*, 1997, **277**, 1971; (*c*) T. W. Odom, J. L. Huang, P. Kim and C. M. Lieber, *J. Phys. Chem. B*, 2000, **104**, 2794; (*d*) J. Liu, A. G. Rizler, H. Dai, J. H. Hafner, R. K. Bradley, P. G. Boul, A. H. Lu, T. Iverson, K. Shelimov, C. B. Huffman, F. Rodriguez-Macias, Y. S. Shon, T. R. Lee, D. T. Colbert and R. E. Smalley, *Science*, 1998, **280**, 1253.

2 (*a*) S. J. Tans, M. H. Devoret, H. Dai, A. Thess, R. E. Smalley, L. J. Geerligs and C. Dekker, *Nature*, 1997, **386**, 474; (*b*) J. Kong, N. R. Franklin, C. Zhou, M. G. Chapline, S. Peng, K. Cho and H. Dai, *Science*, 2000, **287**, 622; (*c*) S. S. Wong, E. Joselevich, A. T. Wooley, C. L. Cheung and C. M. Lieber, *Nature*, 1998, **394**, 52.

3 T. Rueckes, K. Kim, E. Joselevich, G. Y. Tseng, C. L. Cheung and C. M. Lieber, *Science*, 2000, **289**, 94.

4 P. Calvert, *Nature*, 1999, **399**, 210.

5 (*a*) R. J. Chen, Y. Zhang, D. Wang and H. Dai, *J. Am. Chem. Soc.*, 2001, **123**, 3838; (*b*) F. Belavoine, P. Schultz, C. Richard, V. Mallouh, T. W. Ebbesen and C. Mioskowski, *Angew. Chem., Int. Ed.*, 1999, **38**, 1912; (*c*) W. Huang, S. Taylor, K. Fu, Y. Lin, D. Zhang, T. W. Hanks, A. M. Rao and Y.-P. Sun, *Nano Lett.*, 2002, **2**, 311; (*d*) M. Shim, N. Wong Shi Kam, R. J. Chen, Y. Li and H. Dai, *Nano Lett.*, 2002, **2**, 285; (*e*) B. F. Erlanger, B.-X. Chen, M. Zhu and L. Brus, *Nano Lett.*, 2001, **1**, 465; (*f*) M. P. Mattson, R. C. Haddon and A. M. Rao, *J. Mol. Neurosci.*, 2000, **14**, 175.

6 (*a*) J. Chen, M. A. Hamon, H. Hu, Y. Chen, A. M. Rao, P. C. Eklund and R. C. Haddon, *Science*, 1998, **282**, 95; (*b*) Y. Chen, G. Chen, H. Hu, M. A. Hamon, M. E. Itkis and R. C. Haddon, *Chem. Phys. Lett.*, 1999, **299**, 532; (*c*) S. Niyogi, M. A. Hamon, H. Hu, B. Zhao, P. Bhowmik, R. Sen, M. E. Itkis and R. C. Haddon, *Acc. Chem. Res.*, 2002, **35**, Web Release Date: 15 October 2002.

7 (*a*) Y. Lin, A. M. Rao, B. Sadanadan, K. E. A. Kenn and Y. Sun, *J. Phys. Chem. B*, 2002, **106**, 1294; (*b*) W. Zhao, C. Song and P. E. Pehrsson, *J. Am. Chem. Soc.*, 2002, **124**, 12418.

8 (*a*) M. J. O'Connell, P. Boul, L. Ericson, C. Huffman, Y. Wang, E. Haroz, C. Kuper, J. Tour, K. D. Ausman and R. E. Smalley, *Chem. Phys. Lett.*, 2001, **342**, 265; (*b*) B. Z. Tang and H. Xu, *Macromolecules*, 1999, **32**, 2569; (*c*) J. Chen, H. Liu, W. A. Weimer, M. D. Halls, D. H. Waldeck and G. C. Walker, *J. Am. Chem. Soc.*, 2002, **124**, 9034.

9 (*a*) J. L. Bahr, J. Yang, D. V. Kosynkin M. J. Bronikowski, R. E. Smalley and J. M. Tour, *J. Am. Chem. Soc.*, 2001, **123**, 6536; (*b*) M. Holzinger, O. Vostrowsky, A. Hirsch, F. Hennrich, M. Kappes, R. Weiss and F. Jellen, *Angew. Chem., Int. Ed.*, 2001, **40**, 4002; for recent reviews, see (*c*) A. Hirsch, *Angew. Chem., Int. Ed.*, 2002, **41**, 1853; (*d*) J. L. Bahr and J. M. Tour, *J. Mater. Chem.*, 2002, **12**, 1952.

10 V. Georgakilas, K. Kordatos, M. Prato, D. M. Guldi, M. Holzinger and A. Hirsch, *J. Am. Chem. Soc.*, 2002, **124**, 760.

11 A. Star, D. Steuerman, J. R. Heath and J. F. Stoddart, *Angew. Chem., Int. Ed.*, 2002, **41**, 2508.

12 M. J. O'Connell, P. Boul, L. Ericson, C. Huffman, Y. Wang, E. Haroz, C. Kuper, J. Tour, K. D. Ausman and R. E. Smalley, *Chem. Phys. Lett.*, 2001, **342**, 265.

13 M. Sano, A. Kamino, J. Okamura and S. Shinkai, *Langmuir*, 2001, **17**, 5125.

14 F. Pompeo and D. E. Resasco, *Nano Lett.*, 2002, 2, 369.

15 M. G. C. Kahn, S. Banerjee and S. S. Wong, *Nano Lett.*, 2002, in press.

16 K. Kordatos, T. Da Ros, S. Bosi, E. Vazquez, M. Bergamin, C. Cusan, F. Pellarini, V. Tomberli, B. Baiti, D. Pantarotto, V. Georgakilas, G. Spalluto and M. Prato, *J. Org. Chem.*, 2001, **66**, 4915.

17 V. K. Sarin, S. B. H. Kent, J. P. Tam and R. B. Merrifield, *Anal. Biochem.*, 1981, **117**, 147.

18 E. Kaiser, R. L. Colescott, C. D. Bossinger and P. I. Cook, *Anal. Biochem.*, 1970, **34**, 595.

19 M. R. Bendall, D. M. Dodrell and D. T. Pegg, *J. Am. Chem. Soc.*, 1981, **103**, 4603.